確実な塊根形成のためのサツマイモ栽培法

誌名	生物環境調節
ISSN	05824087
巻/号	424
掲載ページ	p. 259-266
発行年月	2004年12月

農林水産省 農林水産技術会議事務局筑波産学連携支援センター
Tsukuba Business-Academia Cooperation Support Center, Agriculture, Forestry and Fisheries Research Council Secretariat

Original Paper Environ. Control in Biol., **42**(4), 259-266, 2004

A Cultivation Method to Ensure Tuberous Root Formation in Sweetpotatoes (*Ipomoea batatas* (L.) Lam.)

Toshihiko EGUCHI and Satoshi YOSHIDA

Biotron Institute, Kyushu University, Fukuoka 812-8581, Japan

(Received January 5, 2004)

Sweetpotato (*Ipomoea batatas* (L.) Lam.) research requires a cultural technique that ensures tuberous root formation for elucidation of the morphogenesis mechanism. Single-root cuttings were grown in a hydroponic system, where the basal part of the root was exposed to the air and the distal part was immersed in nutrient solution. In all of the plants grown in the system, the nodal root swelled in the aerial space, and the thickened part revealed the anatomical characteristics of the tuberous root. The formation of the storage organ occurred earlier in initially thicker roots. After the storage organ initiation, number of leaves in the cuttings affected the thickening growth of the tuberous root.

Keywords: hydroponics, *Ipomoea batatas*, sweetpotato, tuberous root formation

INTRODUCTION

A tuberous root, or a storage root, of sweetpotato (*Ipomoea batatas* (L.) Lam.) is a root having a region of localized thickening (Lowe and Wilson, 1974), wherein the stele enlarges along with the root swelling (Togari, 1950). Several number of adventitious roots emerge from a nodal position of the stem, the roots that are called nodal roots, when stem-cuttings of sweetpotato are planted in soil. Usually some of the roots develop to tuberous roots in the field cultivation. In *Solanum tuberosum* L., there is a cultivation technique to obtain a micro-tuber on single-node cuttings (Duncan and Ewing, 1984; Hendriks et al., 1991). This technique has contributed to the recent advance in the elucidation of the exogenous and endogenous conditions required for the tuber formation (Balamani et al., 1986; Garner and Blake, 1989; Vreugdenhil et al., 1998; Xu et al., 1998; Segreeva et al., 2000). On the other hand, sweetpotato researchers have not obtained such technique to regulate the tuberous root formation yet. A mechanism of the tuberous root formation has been scarcely elucidated, although anatomical changes during the tuberous root development had been detailed (McCormick, 1916; Ogura, 1945a, b; Togari, 1950; Kokubu, 1973; Wilson and Lowe, 1973). Therefore, the following two steps are necessary factors to understand the formation mechanism: 1) development of the cultivation technique to ensure the formation of the storage organ in a specific part of a specific root; 2) search of exogenous and/or endogenous factors that are required for the tuberous root formation. The objective of this study is to realize the first step.

We already found that the sand-cultured sweetpotato with one nodal root inevitably forms a tuberous root regardless of the root temperature in the range of 20-32°C (Eguchi et al., 1994). This cultivation ensures the tuberous root formation in the interested root, whereas it

Corresponding author: Toshihiko Eguchi, fax: +81-92-642-3069,
e-mail: egut@agr.kyushu-u.ac.jp

is difficult to know where the swelling occurs in the root beforehand. Furthermore, changes in shape of the root are difficult to be observed. On the other hand, we can easily observe the root status in a soilless culture, and the root environments such as temperature or nutrient condition are also easily controlled. However, there has been no positive evidence that sweetpotato root can develop to tuberous root within the nutrient solution. The report by Noguchi and Sugawara (1940), which was published more than 60 years ago, gave us a hint for sweetpotato hydroponics that ensure the tuberous root formation. They enclosed a part of the stem in a dark box where the upper layer was the aerial space and the nutrient solution layer was in the bottom. In the box, a number of nodal roots emerged from the stem, and some of the roots successfully formed the storage organ in the aerial space. Based on that idea, we made a small hydroponic system where the root environment was divided into two layers comprised of the aerial space for the root swelling and the nutrient solution layer for uptake of water and nutrients. Single-root plants were certainly able to form the tuberous root in the system. The present paper reports the cultivation method that ensures the tuberous root formation.

MATERIALS AND METHODS

Plant materials. Rooted stem-cuttings of sweetpotato cultivars 'Narutokintoki' and 'Koganesengan' were used as plant materials. Figure 1A shows a procedure for preparation of a material plant. The lowest node of a stem-cutting with three nodes and two leaves was inserted in vermiculite, and the cutting was rooted for 10 days in a phytotron at 25±1°C and 70±5%RH. The single nodal root of about 25 cm length was kept in the plant by excising all other roots. The following four types of material plants were prepared : two leaves plant with the single nodal root of relatively large diameter (about 1.5 mm), T-L ; two leaves plant with the single nodal root of small diameter (about 1 mm), T-S ; single leaf plant with the single nodal root of large diameter, S-L ; single leaf plant with the single nodal root of small diameter, S-S. The difference in width among such young roots is already determined when their primordia are formed (Togari, 1950).

Cultivation. Figure 1B shows a schematic diagram of a hydroponic box. Single-root plants were transplanted in the hydroponic box, which consisted of the nutrient solution layer of 60 mm depth and the aerial space of 90 mm height above the nutrient solution. A porous plastic tube (31 mm inner diameter×90 mm height, about 68 ml inner volume) was stood in

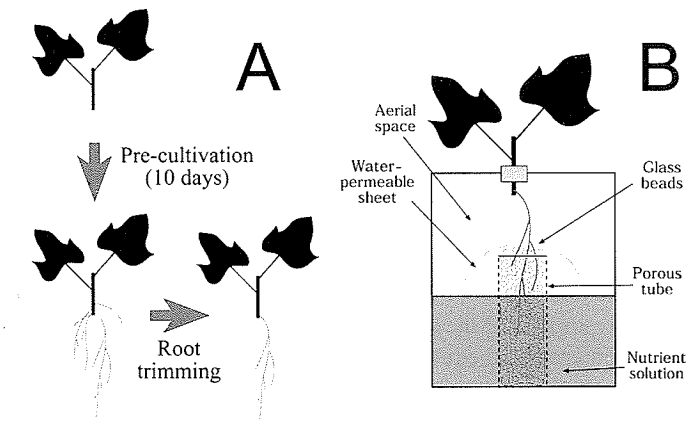

Fig. 1 Schematic diagrams of preparation of a material plant (A) and a hydroponic box (B).

the nutrient solution layer, and the inside wall of the tube was covered with water-permeable sheet (Root barrier sheet, Toyobo, Japan) for confining root elongation within the tube, because vigorous elongation of fibrous root is appeared to depress the root swelling (Eguchi et al., 2003). The nodal root inserted into the tube was held by filling about 35 ml of glass beads (5 mm diameter) within the tube. Glass beads may also have some inhibitory effects on fibrous root elongation. The shoot environment was controlled at an air temperature of 28± 0.5°C, relative humidity of 70±3%, PPFD of 250 μmol m^{-2} s^{-1} and a photoperiod of 12 h (0700-1900 h). Temperature of nutrient solution was controlled at 28±0.5°C by circulating the solution between the hydroponic box and the tank placed in the temperature controlled water bath at a flow rate of 350 ml m^{-1}. Six plants for each type of 'Narutokintoki' and six plants of T-S type 'Koganesengan' were grown in the hydroponic system for 20 days. The maximum diameter of the root was measured at 5 days intervals. The stele diameter of the thickest part was measured at the end of the cultivation. In addition, 10 plants of T-S type 'Narutokintoki' were cultivated for 20 days in a deep-flow-technique (DFT) hydroponic system in which the nodal root was entirely submerged in the nutrient solution. Root diameter of the root-submerged T-S plants was measured at the start and the end of the DFT hydroponics.

Microscopic observation. Anatomical features of the nodal root at the start and the end of 20 days cultivation were observed. Hand-cut cross-sections were obtained from the basal part (4 cm distant from the joint) of the nodal root at the initial state. After 20 days cultivation in the hydroponic system, cross-sections of the thickest root part were obtained from all types of plants. Sections were stained with 0.02% Toluidine Blue or 2% Phloroglucinol and HCl, and observed with optical microscopes (OPTIPHOT, Nikon, Japan, and SMZ1000, Nikon). Cross-sections were also obtained from S-S type 'Narutokintoki' when the root width reached 2, 3 and 5 mm, and used to observe the anatomical changes during the root thickening.

RESULTS

Figure 2 shows changes in root shape of T-L type 'Narutokintoki' during 20 days cultivation in the hydroponic box. The nodal root uniformly thickened in the aerial space at 5 days after transplanting (DAT). At 10 DAT, the root unevenly thickened as the middle of the air-exposed root part became narrow spindle-shape. Thereafter the spindle-shaped root grew thicker. Figure 3A shows changes of the maximum diameter of the nodal root in 'Narutokintoki' during 20 days cultivation. The nodal root increased the width with time in all plants. Those roots showed uniform thickening until 5 DAT in T-L and S-L, and until 10 DAT in T-S and S-S, at which their diameter reached about 2 mm. The spindle-shaped root was confirmed at 10 DAT in T-L and S-L, and at 15 DAT in T-S and S-S, when their diameter reached about 4 mm. Time-course pattern of the root diameter in S-L was similar to the pattern in T-L until 10 DAT. Thereafter, the nodal roots of T-L became thicker than the S-L roots. Changes in root diameter of S-S were also similar to that of T-S until 15 DAT. Time course pattern of T-S type 'Koganesengan' agreed with that of T-S type 'Narutokintoki' (Fig. 3B).

Figure 4 shows anatomical changes in the root during the root thickening. At the initial state, the basal part of the root represented the following characteristics : fibrillose root surface, adjacency of protoxylem to central metaxylem, and indistinct primary cambium. Although the material plants differed in root width that varied around 1 mm to nearly 1.5 mm, those characteristics were identical for all roots regardless of their width. The nodal root uniformly increased width to 2 mm in diameter in the aerial space of the hydroponic box. In the

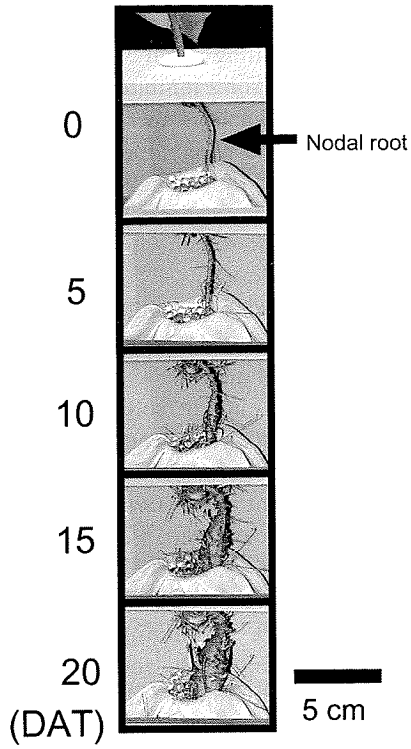

Fig. 2 Nodal root thickening in the aerial space of the hydroponic box during 20 days cultivation.

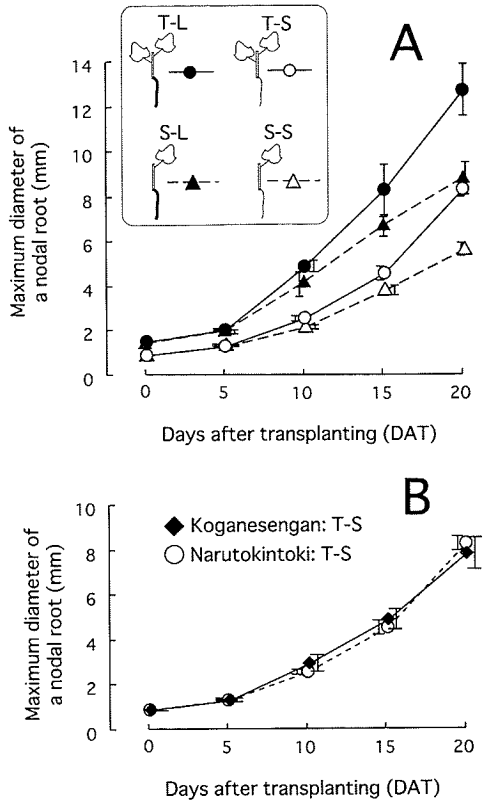

Fig. 3 Changes in the maximum diameter of the nodal root of 'Narutokintoki' (A) and 'Koganesengan' (B). Means of six plants are plotted with standard errors. T-L, plant with two leaves and single root of relatively large diameter (1.5 mm); T-S, two leaves and single root of small diameter (1 mm); S-L, single leaf and single root of large diameter; S-S, single leaf and single root with small diameter.

thickened region of the root, the primary cambium ring was formed in the periphery of the stele, and meristem occurred within the stele. Parenchyma separated protoxylem from central metaxylem in the stele. The root width was being uneven along the longitudinal axis when the root grew to 3 mm width. At this stage, a lot of secondary cambia was observed in the stele where the interstitial parenchyma further increased the area. In the root of 5 mm width, the stele occupied large area of the thickened root, and xylem vessels were dispersedly located in the stele.

Table 1 shows maximum diameter of the nodal root and frequency of the secondary cambium formation within the stele at 20 DAT in 'Narutokintoki.' Data of the root-submerged T-S plants was also listed in this table. The root diameter at 20 DAT reached 5.65 mm in S-S, 8.80 mm in S-L, 8.48 mm in T-S and 12.72 mm in T-L, which corresponded to about 6- and 8.5-fold of the initial diameter for single leaf plants (S-L and S-S) and two leaves plants (T-L and T-S), respectively. The stele diameter occupied more than 70% of the root diameter and secondary cambium already formed within the stele in all of those four types.

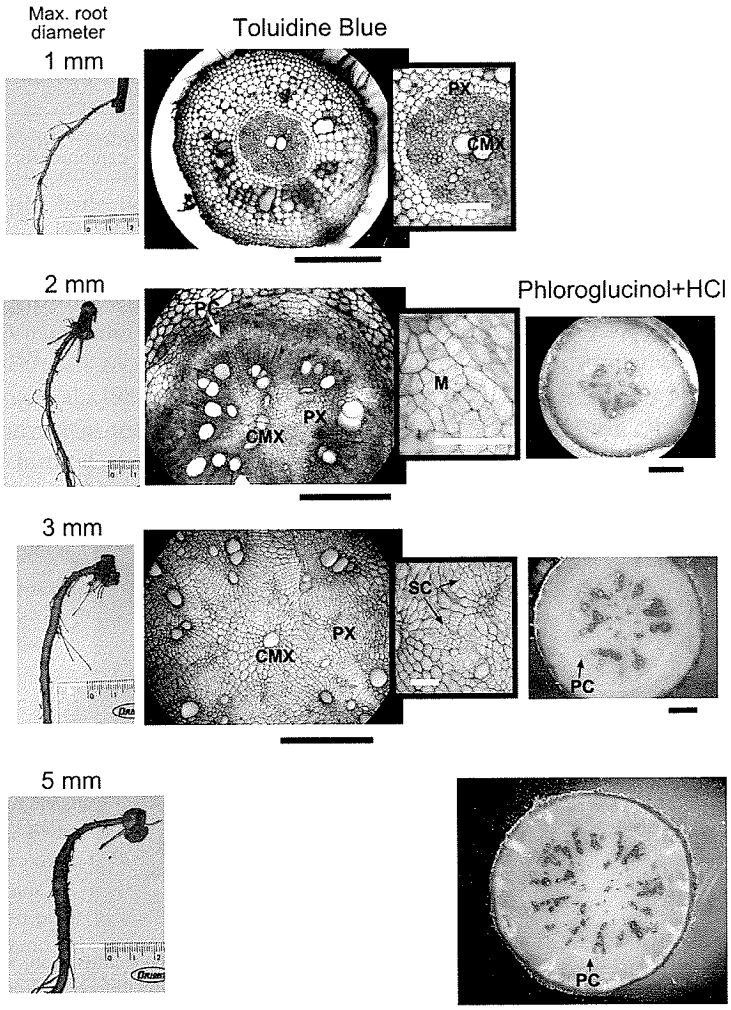

Fig. 4 Anatomical changes during the root thickening in S-S type 'Narutokintoki.' Hand-cut cross-sections of the nodal root were stained with 0.02% Toluidine Blue or Phloroglucinol and HCl. Xylem vessels and other lignified cells were stained blue with Toluidine Blue, and red with Phloroglucinol+HCl. Primary cambium was observed as a yellowish ring in the stereoscopic image of cross-sections stained with Phloroglucinol+HCl. Black bars indicate 0.5 mm and white bars indicate 0.1 mm. PX, protoxylem; CMX, central metaxylem; PC, primary cambium; M, meristem; SC, secondary cambium.

On the other hand, the nodal root diameter of the root-submerged T-S plants only became 2-fold thicker than the initial value, and there was no part showed spindle-shape (Fig. 5). The stele diameter of the root-submerged T-S plants was nearly the half of the root diameter, and secondary cambium was not observed within the stele. The central region of the stele was heavily lignified (Fig. 5).

DISCUSSION

The nodal root increased the width in the aerial space of the hydroponic system in all

Table 1 Diameter of the thickest region in the nodal root of 'Narutokintoki' after 20 days cultivation, percentage of the stele diameter in the region, and frequency of the plant having the secondary cambium within the stele. Values of diameter are means of six plants for T-L, T-S, S-L and S-S, and 10 plants for root-submerged T-S.

Plant type	Diameter of the nodal root, mm	Stele diameter/ root diameter, %	Frequency of the secondary cambium formation, %
T-L	12.72 (8.7)[z]	83.8	100
T-S	8.48 (8.5)	75.9	100
S-L	8.80 (6.1)	75.5	100
S-S	5.65 (5.7)	70.5	100
Root-submerged T-S	2.15 (2.2)	46.5	0

[z] Relative value to the initial diameter of the nodal root.

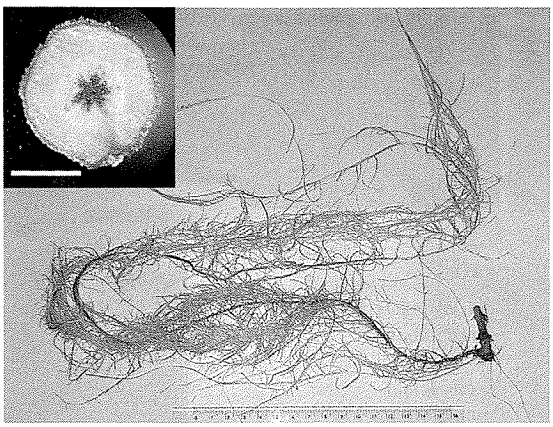

Fig. 5 A nodal root of the root-submerged T-S 'Narutokintoki' after 20 days cultivation, and a stereoscopic image of hand-cut cross-section of the basal part stained with Phloroglucinol+HCl. A white bar indicates 1 mm.

plants (Figs. 2 and 3). The stele enlarged in the thickened root, and secondary cambia were formed within the stele (Table 1). Heavy lignification was not observed even in the root of S-S plants that showed the least thickening (Fig. 4). Meristem such as the secondary cambium within the stele contributes to the localized increase in width of the nodal root during the tuberous root development (McCormick, 1916; Ogura, 1945a, b; Togari, 1950; Kokubu, 1973; Wilson and Lowe, 1973). The enlarged stele without heavy lignification represents a morphological characteristic of tuberous root (Togari, 1950). Thus, the thickened root part in the aerial space was confirmed to be the tuberous root. The storage organ formation was initiated before the root thickening became uneven, because meristem within the stele already formed in the uniformly thickened root of 2 mm width (Fig. 4). The onset of the tuberous root formation occurred regardless of the number of leaves in the single-root plants, while the onset time delayed in initially thinner roots (Fig. 3A). The thickening growth thereafter may be affected by the ability of photoassimilate supply to the roots, since the root width of two leaves plants became thicker than that of single leaf plants. Genetic variation of the root thickening was not observed between 'Narutokintoki' and 'Koganesengan' (Fig. 3B). In

addition to those results, we confirmed that the nodal root was not able to form the storage organ when the root completely submerged in the nutrient solution (Table 1 and Fig. 5).

Using the cultivation procedure of this study, a specific part of the nodal root inevitably forms the storage organ. Noguchi and Sugawara (1940) demonstrated first that the swelling can occur in the air-exposed part of the nodal root, but it took about one month from the root emergence to the onset of the swelling. In this study, T-L plants required only 10 days cultivation in the hydroponic system for tuberous root formation, which was about 20 days after the root emergence. In potato research, *in vitro* culture of single-node cuttings has been used for elucidation of exogenous and endogenous conditions required for the tuber initiation and the tuber growth thereafter (Hussey and Stacey, 1984 ; Balamani et al., 1986 ; Garner and Blake, 1989 ; Vreugdenhil et al., 1998 ; Xu et al., 1998 ; Segreeva et al., 2000). This technique also helps researchers to analyze gene expression during the tuber formation (Rosahl et al., 1986 ; Stiekema et al., 1988 ; Visser et al., 1994 ; Raíces et al., 2001). In sweetpotato research, there is no convenient technique which control the tuberous root formation, but this study shows a new method at which the tuberous root surely formed in the aerial space of the hydroponic box. This may contribute the sweetpotato study to optimize exogenous and endogenous conditions related to the tuberous root formation.

This work was supported by a Grant-in-Aid for Scientific Research (c)(2) (no. 15580224) from the Japan Society for the Promotion of Science.

REFERENCES

Balamani, V., Veluthambi, K., Poovaiah, B. W. 1986. Effect of calcium on tuberization in potato (*Solanum tuberosum* L.). Plant Physiol. **80** : 856-858.

Duncan, D. A., Ewing, E. E. 1984. Initial anatomical changes associated with tuber formation on single-node potato (*Solanum tuberosum* L.) cuttings. Ann. Bot. **53** : 607-610.

Eguchi, T., Kitano, M., Eguchi, H. 1994. Effect of root temperature on sink strength of tuberous root in sweet potato plants (*Ipomoea batatas* Lam.). Biotronics **23** : 75-80.

Eguchi, T., Kitano, M., Yoshida, S., Chikushi, J. 2003. Root temperature effects on tuberous root growth of sweetpotato (*Ipomoea batatas* Lam.) —Direct and indirect effects of temperature—. (Japanese text with English abstract) Environ. Control in Biol. **41** : 43-49.

Garner, N., Blake, J. 1989. The induction and development of potato microtubers *in vitro* on media free of growth regulating substances. Ann. Bot. **63** : 663-674.

Hendriks, T., Vreugdenhil, D., Stiekema, W. J. 1991. Patatin and four proteinase inhibitor genes are differentially expressed during potato tuber development. Plant Mol. Biol. **17** : 385-394.

Hussey, G., Stacey, N. J. 1984. Factors affecting the formation of 'in vitro' tubers of potato (*Solanum tuberosum* L.). Ann. Bot. **53** : 565-578.

Kokubu, T. 1973. Thremmatological studies on the relationship between the structure of tuberous root and its starch accumulating function in sweet potato varieties. (Japanese text with English summary) Bull. Fac. Agr., Kagoshima Univ. **22** : 1-126.

Lowe, S. B., Wilson, L. A. 1974. Comparative analysis of tuber development in six sweet potato (*Ipomoea batatas* (L.) Lam.) cultivars. 1. Tuber initiation, tuber growth and partition of assimilate. Ann. Bot. **38** : 307-317.

McCormick, F. A. 1916. Notes on the anatomy of the young tuber of *Ipomoea batatas* Lam. Bot. Gaz. **61** : 388-398.

Noguchi, Y., Sugawara, T. 1940. A study on mechanism of tuberous root formation in sweet potatoes. (Japanese text) Agric. Hortic. **15** : 1-8.

Ogura, K. 1945a. Anatomical studies on tuberous root formation in sweet potatoes [1]. (Japanese text) Agric. Hortic. **20** : 331-334.

Ogura, K. 1945b. Anatomical studies on tuberous root formation in sweet potatoes [2]. (Japanese text)

Agric. Hortic. **20**: 381-383.
Raíces, M., Chico, J. M., Téllez-Iñón, M. T., Ulloa, R. M. 2001. Molecular characterization of StC-DPK1, a calcium-dependent protein kinase from *Solanum tuberosum* that is induced at the onset of tuber development. Plant Mol. Biol. **46**: 591-601.
Rosahl, S., Eckes, P., Schell, J., Willmitzer, L. 1986. Organ-specific gene expression in potato: isolation and characterization of tuber-specific cDNA sequences. Mol. Gen. Genet. **202**: 368-373.
Segreeva, L. I., de Bruijin, S. M., Koot-Gronsveld, E. A. M., Navratil, O., Vreugdenhil, D. 2000. Tuber morphology and starch accumulation are independent phenomena: evidence from *ipt*-transgenic potato lines. Physiol. Plant. **108**: 435-443.
Stiekema, W. J., Heidekamp, F., Dikse, W. G., Van Beckum, J., De Haan, P., Ten Bosch, C., Louwerse, J. D. 1988. Molecular cloning and analysis of four potato tuber mRNAs. Plant Mol. Biol. **11**: 255-269.
Togari, Y. 1950. A study of tuberous root formation in sweet potato. (Japanese text with English summary) Bull. Nat. Agr. Exp. Station **68**: 1-96.
Visser, R. G. F., Vreugdenhil, D., Hendriks, T., Jacobsen, E. 1994. Gene expression and carbohydrate content during stolon to tuber transition in potatoes (*Solanum tuberosum*). Physiol. Plant. **90**: 285-292.
Vreugdenhil, D., Boogaard, Y., Visser, R. G. F., de Bruijin, S. M. 1998. Comparison of tuber and shoot formation from *in vitro* cultured potato explants. Plant Cell, Tissue and Organ Culture **53**: 197-204.
Wilson, L. A., Lowe, S. B. 1973. The anatomy of the root system in West Indian sweet potato (*Ipomoea batatas* (L.) Lam.) cultivars. Ann. Bot. **37**: 633-643.
Xu, X., van Lammeren, A. A. M., Vermeer, E., Vreugdenhil, D. 1998. The role of gibberellin, abscisic acid, and sucrose in the regulation of potato tuber formation in vitro. Plant Physiol. **117**: 575-584.

〈和文抄録〉

確実な塊根形成のためのサツマイモ栽培法

江口壽彦・吉田　敏

九州大学生物環境調節センター

サツマイモ塊根の形成機構を解明するために，確実に塊根形成個体を得るための水耕法を確立した．茎挿しで育成した節根1本を有する植物を水耕装置に植え，節根の基部側を空気中に保持し，より先端側を培養液中に浸して栽培したところ，20日間の栽培で実験に用いたすべての個体において，節根の空気中にある部位で局所的な肥大が認められた．この部位は解剖学的な特徴から塊根であると確認された．また，葉数および初期根径の異なる個体を本方法で栽培した結果，初期根径は塊根形成開始時期と関係が深く，葉数も塊根形成開始後の肥大に影響を及ぼすことがわかった．